BLOOD

Obustami 1

a tissue consisting of a variety of cells suspended in a fluid medium called plasma

↓

Main function → a vehicle for the transport of gases, nutrients, metabolic waste products, cells & hormones throughout the body

↓

PLASMA → essentially aqueous solution of inorganic salts which is constantly exchanged with extracellular fluid of body tissues

↓

plasma also contains → **Plasma Proteins** of 3 main types.
- albumins
- globulins
- fibrinogen

} collectively exert a colloid osmotic pressure within the circulatory system which helps to regulate the exchange of aqueous solutions between plasma & ECF

↓

Albumins → constitute the Bulk of plasma Proteins

↓

bind insoluble metabolites such as fatty acids & serve as transport proteins

globulins — includes the antibodies of immune system
transport lipids

Fibrinogen — soluble protein which polymerises to form the insoluble protein fibrin during blood clotting

Blood cell types → 3 major classes
- → Red blood cells (RBCs) (erythrocytes)
- → white blood cells (WBCs) (leucocytes)
- → platelets (thrombocytes)

All are formed in the bone marrow → a process known as **haemopoiesis**

↓

Erythrocytes — involved in transport of O_2 / CO_2
function exclusively within the vascular system

Leucocytes — important part of defence & immune system
act mainly OUTSIDE blood vessels in the TISSUES

platelets — play a vital role in the control of bleeding (haemostasis) by a) plugging the defects in blood vessels b) activating the blood clotting cascade

Methods used to study blood & bone marrow

→ to make a smear on a glass slide

After fixation stained by a **Polychromatic Stain** — Giemsa, Wright, leishman

↓

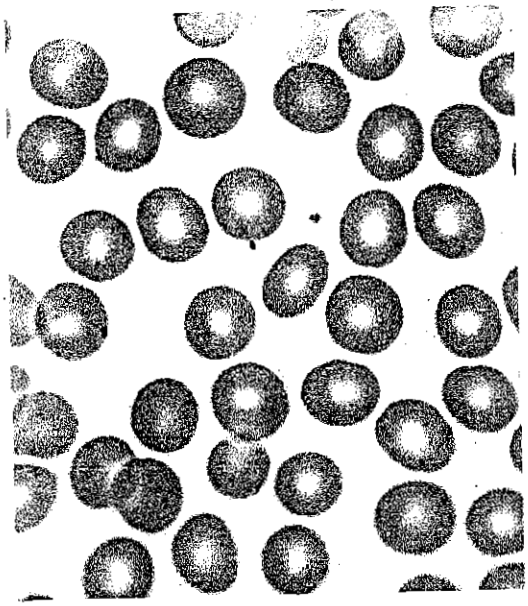
(4) distinctive staining characteristics (according to the affinity of the various cellular organelles to the different stains employed)

(a) Basophilia (deep blue): affinity for the basic dye methylene blue → characteristic of DNA in nucleus & RNA in the cytoplasm e.g Ribosomes

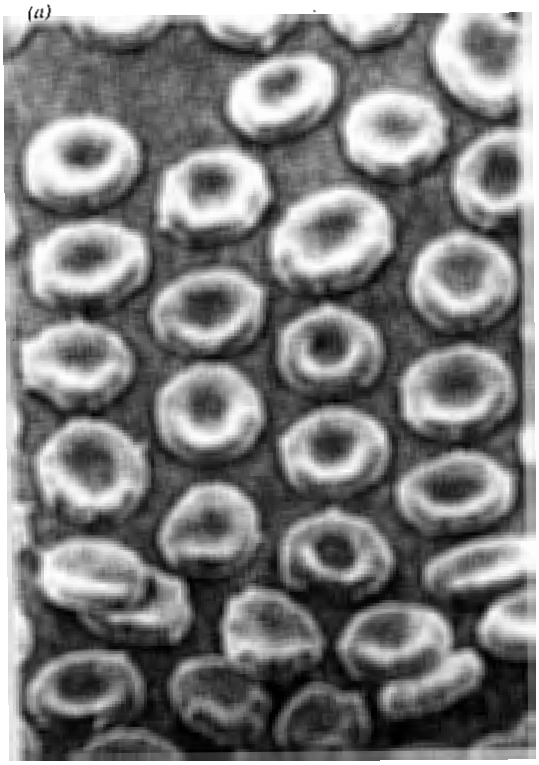
(b) azurophilia (purple) → affinity for azur dyes, typical of lysosomes (azurophilic granules in leucocytes)

(c) eosinophilia (pink) → affinity for acidic dye (eosin)
particular feature for hemoglobin within erythrocytes

(d) neutrophilia (salmon pink / lilac): characteristic of the specific cytoplasmic granules of neutrophil leucocytes (the dye is not of neutral pH)



(a)



Erythrocytes

Giemsa stain
Scanning EM

RBC are Pink biconcave discs that are 7–8 μm in diameter

Pink (eosinophilia / acidophilia) is due to their high content of hemoglobin which is a basic protein

The pale staining of the central region is a result of its biconcave disc shape

provides 20–30% greater surface area than a sphere relative to cell volume → thus significantly enhancing gas exchange

This shape along with the fluidity of the plasma membrane allows erythrocyte to deform readily & thus erythrocytes (average diameter 7.2 μm) are able to pass through the smallest capillaries (3–4 μm in diameter)

Erythrocytes are enclosed by a typical cell membrane → During differentiation in the bone marrow, vast quantities of the iron-containing respiratory pigment haemoglobin are synthesized → Before release into the blood circulation, the erythrocyte nucleus is extruded and by maturity all cytoplasmic organelles degenerate

The fully differentiated erythrocyte consists merely of an outer plasma membrane enclosing haemoglobin and the limited number of enzymes necessary for maintenance of plasma membrane integrity & gas transport functions

Immediatly beneath the plasma membrane is a MESHWORK of proteins forming a cytoskeleton → responsible in part for the biconcave shape of erythrocytes

Number of erythrocytes — in ♂ 4.500.000 — 6.000.000/cu. mm
in ♀ 3.800.000 — 5.000.000/ "

Lifespan = 120 days → without organelles → erythrocytes are unable to replace necessary enzyme & membrane proteins

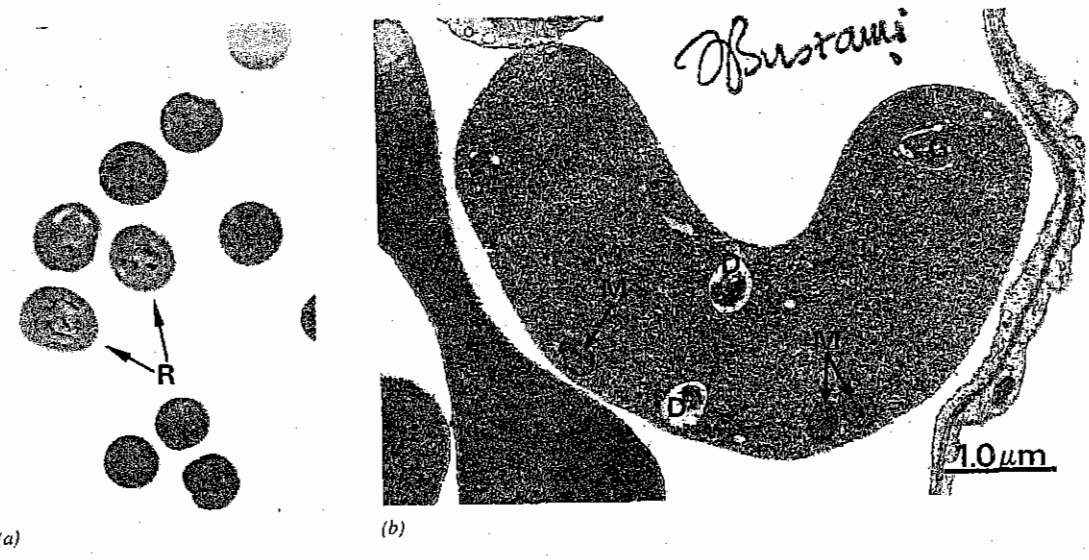


Fig. 3.3 Reticulocytes (a) Cresyl blue/eosin × 1200 (b) EM × 16 000

Reticulocytes are the immature form in which erythrocytes are released into the circulation from the bone marrow

They still contain sufficient { mitochondria, ribosomes, Golgi } to complete cytoskeleton & the remaining 20% of hemoglobin synthesis

Final maturation into erythrocytes occurs within 24 - 48 hours of release

The rate of release of reticulocytes into the circulation generally equals the rate of removal of spent erythrocytes by the spleen & liver → Reticulocytes constitute slightly less than 1% of circulating red blood cells

In routinely stained blood smears → Reticulocytes cannot be easily distinguished from mature erythrocytes.

When fresh blood is incubated with basic dye → brilliant cresyl blue → a blue-stained Reticular precipitate is formed in the reticulocytes due to the interaction of the dye with ribosomal RNA remnants → the technique is called supravital staining.

Reticulocytosis

Abnormalities of Erythrocytes

A change from the normal size shape or staining properties of erythrocytes are important indicator of disease. However, some of these abnormalities may be found in healthy individuals.

Anisocytosis → abnormal VARIATIONS in the SIZE of the red cells which may be Macrocytes (larger than normal) or microcytes (smaller than normal)

Poikilocytosis → abnormal Shape of RBCs

- the cells may show blunt / pointed projections from their surface
- Crenation of Red cell → if normal cells are placed in a hypertonic solution → water is abstracted from the cells leaving it shrunken & bearing numerous projections from its surface
- one of the most severe changes in shape occurs during Sickling of RBCs in Sickle cell anaemia where the erythrocytes take on the form of crescents.

Hypochromia → denotes a decrease in the intensity of staining → indicates a decreased amount of haemoglobin
→ frequently accompanies microcytosis
⇒ hypochromic microcytic anaemia

Howell-Jolly bodies → nuclear fragments left over from the nucleated precursors of the red cell

{ An increase in the number of Reticulocytes is considered an abnormality but the cell itself is not an abnormal erythrocyte

White blood cells

5 types of leucocyte are normally present in the circulation → depending on their nuclear shape & cytoplasmic granules

Bustami

granulocytes
* Neutrophils
* eosinophils
* basophils

Mononuclear leucocytes
* lymphocytes
* monocytes

Granulocytes
- so named → their prominent cytoplasmic granules
- Each cell → type-specific granules ← N E B
- Single multilobed nucleus (old concept → multinuclear)

Polymorphonuclear leucocyte (Polymorph) ? the multilobed nucleus may assume many morphological shapes !!
→ mostly used to refer to neutrophils !!

Lymphocytes & monocytes → have Non-lobulated nuclei (Mononuclear !!)
↓
Agranulocytes (old term !!)
↓
< they are not devoid of cytoplasmic granules >

Recall → lymphocytes play a key role in all immune responses → in contrast to the other leucocytes their activity is always directed against specific foreign agents

Neutrophils & monocytes → highly phagocytic & engulf microorganisms, cell debris → in a Non-specific manner

all the leucocytes perform their functions in the tissues & merely use the blood as a vehicle for transit between sites of formation → storage → activity

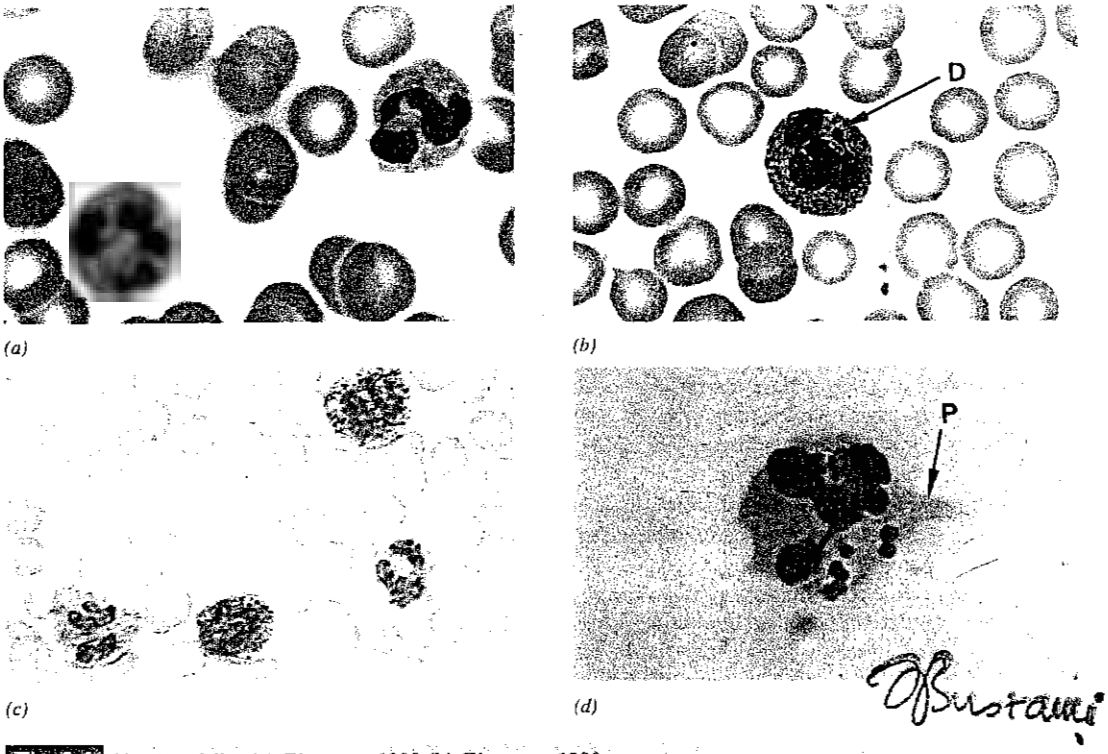


Fig. 3.4 Neutrophils (a) Giemsa × 1200 (b) Giemsa × 1200
(c) Histochemical method for alkaline phosphatase × 800 (d) Giemsa × 2400

Neutrophils → the most common type of leucocyte in blood & constitute 40–75% of circulating leucocytes

→ characterized by the shape of the nucleus which contains small lobes connected by thin filaments

↓

When mature → there are usually 5 lobes connected by fine strands of nuclear material. In less mature neutrophils the nucleus is less lobulated.

→ Neutrophil of ♀ → the condensed quiescent X-chromosom or barr body exists in the form of a small drumstick-shaped appendage of the nuclear lobes ⇒ visible in about 3% of neutrophils in Peripheral blood.

→ Cytoplasm contains 2 types of granules:

- The most numerous are the specific granules (barely visible by light microscopy) which are small and take on a pinkish colour. These granules contain Lysozyme an enzyme complex that act against bacterial cell wall, the protein Phagocytin is present and also has bactericidal activity

The azurophilic granules are less numerous somewhat larger and stain a reddish purple. (8/A)

They are modified large lysosomes and contain the usual lysosomal hydrolases and a number of bactericidal agents including myeloperoxidase → this can be demonstrated by the peroxidase stain and is used as a marker for the primary (azurophilic) granules and for identification of leukaemia arising in neutrophil precursors

Functions of Neutrophils

Being highly motile & phagocytic → their principle function is in the (Acute inflammatory response) to tissue damage where they ingest & destroy damaged tissue & invading bacteria

↓

Neutrophils in the circulation are attracted by the presence of organisms, particularly bacteria → This process is mediated by chemotactic factors (chemotaxins) released from damaged tissue & generated by the interaction of antibodies with antigens on the surface of micro-organisms.

↓

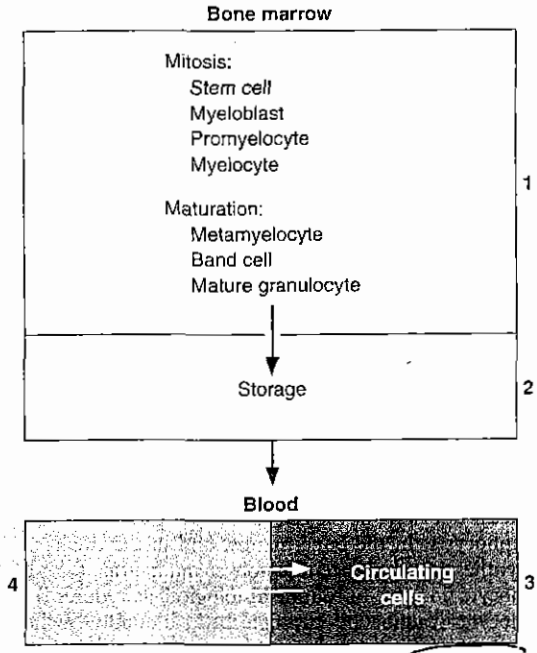
The COATING of organisms with antibodies & complement greatly enhances neutrophilic phagocytic activity → opsonisation ←

↓

organisms which do not generate chemotaxins or become opsonised → are relatively resistant to neutrophil phagocytosis & are thus highly pathogenic

Neutrophilia !!

(8)B



1 → Granulopoietic compartment (Bone marrow)
- mitotic part
- maturation part

↓

2 → storage comp. (also in Red marrow)

↓

3 → circulating comp. throughout blood

↓

4 → marginating comp. (postcapillary venules & small veins)

Figure 13–10. **Functional compartments of neutrophils.** Neutrophils exist in at least four anatomically and functionally distinct compartments, the sizes of which are proportional to the number of cells. **(1)** Granulopoietic compartment subdivided into a mitotic part and a maturation part. **(2)** Storage (reserve) compartment, also in red marrow, acts as a buffer system, capable of releasing large numbers of mature neutrophils on demand. **(3)** Circulating compartment throughout the blood. **(4)** Marginating compartment, in which cells temporarily do not circulate, but rather adhere via selectins to vascular endothelial cells of postcapillary venules, particularly in the lungs. The marginating and circulating compartments are of about equal size, and there is a constant interchange of cells between them. The half-life of a neutrophil in these two compartments is less than 10 hours. The granulopoietic and storage compartments together include cells in the first 11 days of their existence and are about 10 times larger than the circulating and marginating compartments.

Neutrophilia (increase in the number of neutrophils in the circulation) ⟹ DOES NOT necessarily imply an increase in neutrophil production

↓

- intense muscular activity
- administration of epinephrine

→ Produce **APPARENT** neutrophilia !!

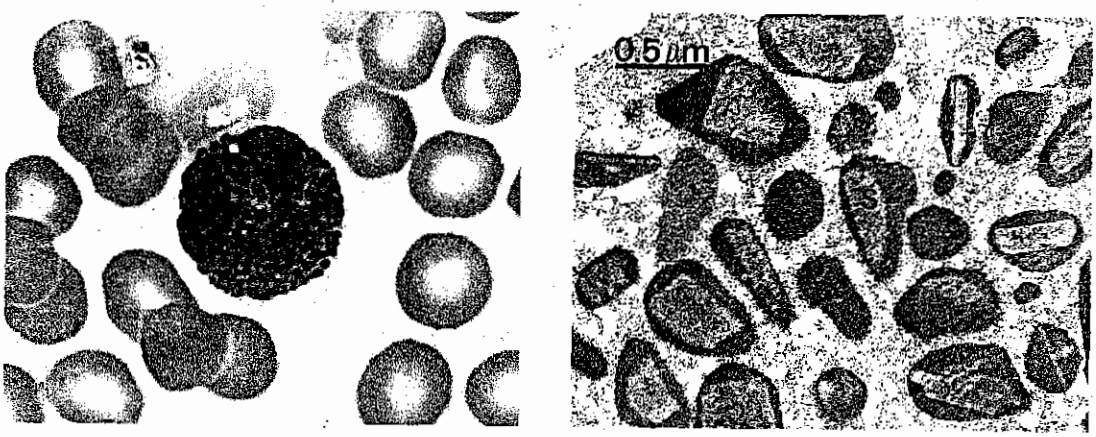
→ Causes neutrophils in the marginating compartment to move into the circulating compartment

glucocorticoids → increase the mitotic activity of neutrophil precursors in the marrow ⟹ increase blood count of neutrophils

Neutrophilia that occurs during bacterial infections

→ increase production of neutrophils ⊕ short duration of these cells in bone marrow

→ Band cells, neutrophilic metamyelocytes & even myelocytes may appear in blood stream (longer duration)



(a) (b)

Fig. 3.6 Eosinophils (a) Giemsa × 1600 (b) Human EM × 25 000 (c) Mouse EM × 20 000 *(opposite)* (d) Rat EM × 25 000 *(opposite)*

Obustami (9)

Eosinophils: account for [1–6%] of leucocytes in circulating blood ⇒ their numbers exhibit a marked diurnal variation, being <greatest in the morning> and least in the afternoon.

↓

→ The eosinophil (12–17 μm) in diameter, is larger than the neutrophil → easily recognized by its large specific granules which are stained Bright red é eosin

→ most cells have bilobed nucleus (often partly obscured by cytoplasmic granules)

⇓

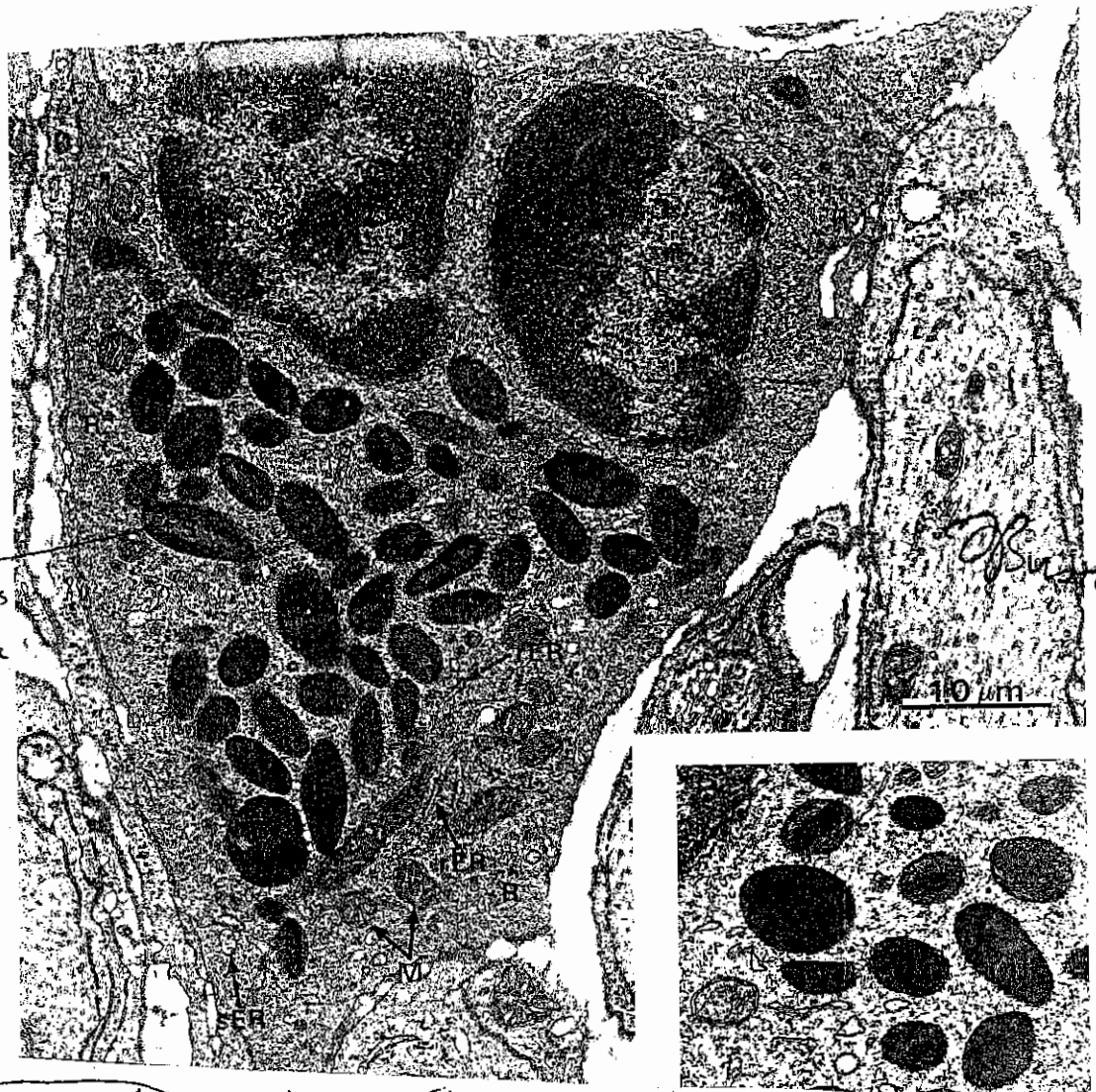
The granules are membrane-bound and shows an internal structure that has been called a crystalloid or internus (In Man it assumes various forms and is translucent)

⇓

The eosinophils granules are lysosomes and contain the usual lysosomal enzymes <including histaminase> → They show a higher content of peroxidase than do the azurophilic granules of neutrophils & lack lysozyme & phagocytin

<Eosinophils are phagocytic cells But less bactericidal than neutrophils, however they have a particular phagocytic affinity for antigen-antibody complex>

All eosinophils have receptors for IgE (important in the destruction of parasites) → this is Not present on neutrophils



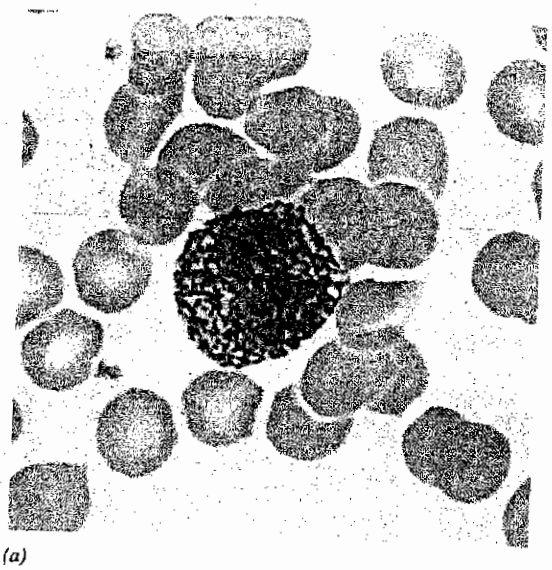
Eosinophils undergo (Chemotaxis) in response to (histamine) & eosinophil chemotactic factor of anaphylaxis (ECF-A) released from basophils & mast cell.

Eosinophils → ameliorate (تخفف) some aspects of hypersensitivity reactions ?? → they neutralize histamine & produce a factor → eosinophil-derived inhibitor which inhibits mast cell degranulation

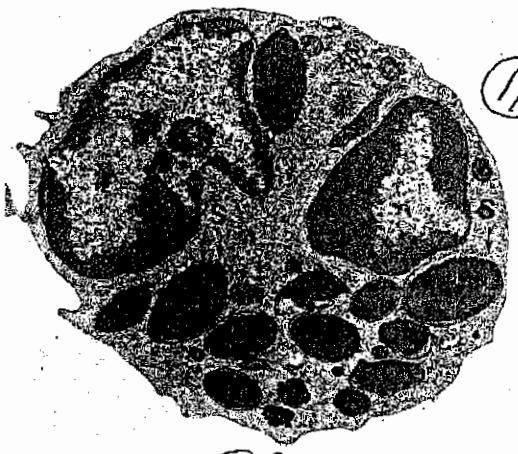
Eosinophilia → ↑ No. of circulating eosinophils are found in ① many types of parasitic disease (defence against parasites is one of their principle functions) ② In some allergic disease e.g hay fever & asthma → their role here ??

From bone marrow → circulation (3-6 hours) → enters ← skin, pulmonary mucosa, GI mucosa → local secretion

Fate & lifespan → unknown !!



(a)



(b)

(11)

Fig. 3.7 Basophils (a) Giemsa × 1500 (b) EM × 10 500

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Basophils → least common leucocyte
→ constitutes less than 1% of leucocytes in circulating blood.
→ lifespan → unknown (few days !!)
→ 14–16 μm in diameter (intermediate between N & E)
→ bilobed nucleus obscured by large densely basophilic (deep blue) specific granules which are larger but fewer in number than those of eosinophils → granules are metachromatic !! they contain proteoglycans. The granules contains heparin, histamine, eosinophil chemotactic factor of anaphylaxis (ECFA)

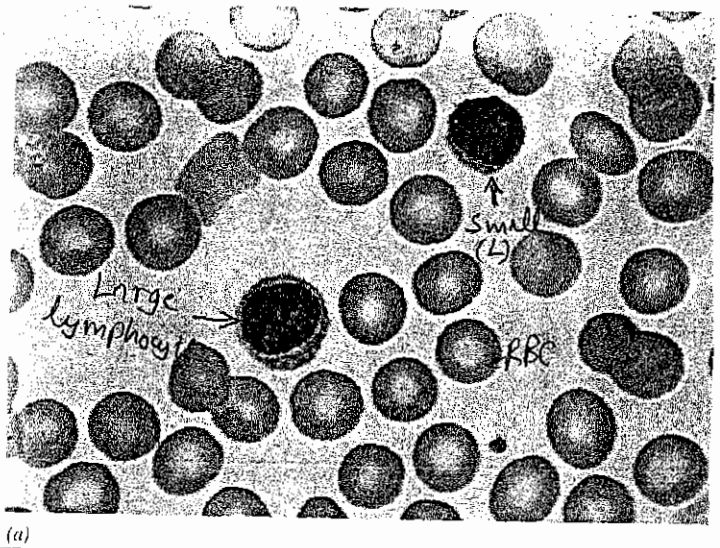
Basophil & mast cells → have membrane receptors highly specific for the Fc segment of IgE (1) which is produced by plasma cells in response to a variety of allergens

(2) Exposure to allergen results in antigen forming bridges between adjacent IgE molecules which triggers rapid exocytosis of granules (degranulation)

⇓

The release of histamine & other mediators is responsible for the so called → immediate hypersensitivity (anaphylactoid) reaction characteristic of
- allergic rhinitis (hay fever)
- some forms of asthma
- urticaria
- anaphylactic shock

Basophils → account for up to 15% of infiltrating cells in allergic dermatitis & skin allograft rejection
→ Cutaneous basophil hypersensitivity



(a)

Lymphocytes

(12)

① the smallest cells in the white cell series (slightly larger than erythrocytes)

② the second most common leucocyte in circulating blood & make up 20–25% of the differential white cell count

③ increased numbers are commonly seen in viral infections

④ characterised by large round densely stained nucleus & a relatively small amount of basophilic non-granular cytoplasm

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⑤ The amount of cytoplasm depends upon state of activity of the lymphocyte ⟹ In circulating blood there is predominance of small inactive lymphocytes (6–9 μm in diameter). Large lymphocytes (9–15 μm) make up about 3% of lymphocytes in peripheral blood ⟹ Represent activated B lymphocytes en route to the tissues where they will become antibody-secreting plasma cells, they also include natural killer cells

↓

In the large lymphocyte → the cytoplasm is readily visible but in the small lymphocyte the cytoplasm is almost too sparse to be seen (contain few mitochondria, rudimentary Golgi apparatus, minimal endoplasmic reticulum but larg. No. of ribosomes → account for basophilia (blue cytoplasm)

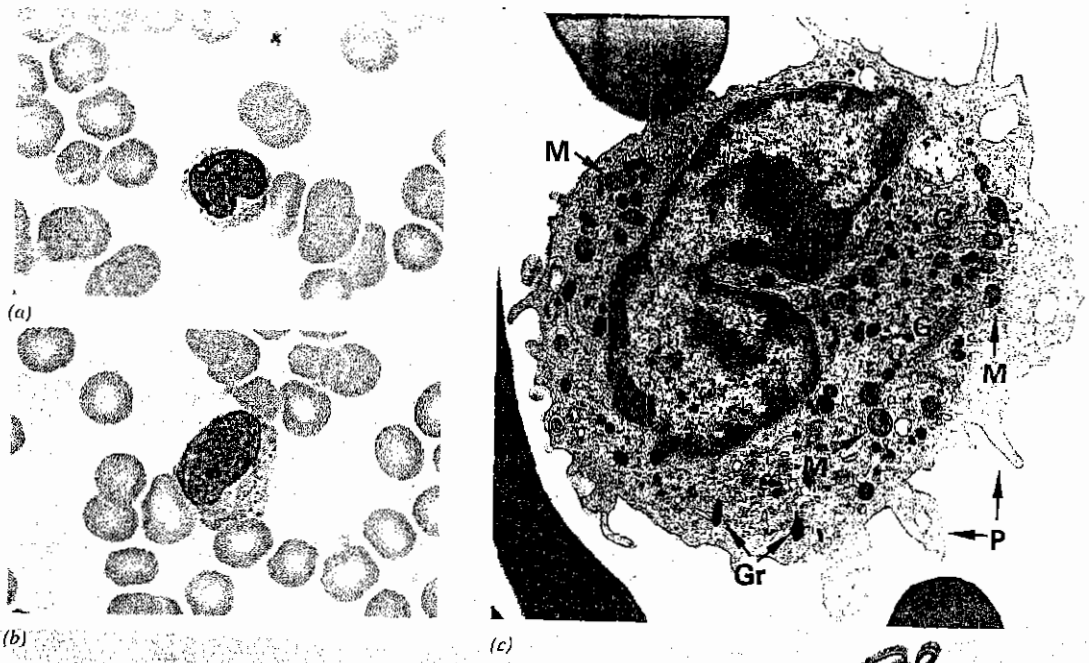

Fig. 3.9 Monocytes (a) Giemsa × 1000 (b) Giemsa × 1000 (c) EM × 20 000

Dr Bustami

Monocytes
- → the LARGEST of white cells (up to 20 μm in diameter)
- → constitute 2 – 10% of Leucocyte in peripheral blood
- → highly motile & Phagocytic
- → Precursor of MACROPHAGES found in peripheral tissues & lymphoid organs

nucleus
- large eccentric stained less intensely than that of other leucocytes
- often with deep indentation (+) 2-3 nucleoli

cytoplasm
- stain pale greyish - blue
- contains small pink-purple lysosomal granules
  - → at EM → primary lysosomes similar to azurophilic granules of neutrophils (contain acid phosphatase & peroxidase)

Monocytes – macrophage system → monocytes migrate to peripheral tissues where they assume the role of macrophages → This has led to the concept of a single functional unit (precursors in bone marrow → circulating monocytes → tissue macrophages)

Included in this system are → Kupffer cells of liver, microglia of CNS, Langerhans cells of the skin, Antigen-presenting cells of lymphoid organs (APC), osteoclasts of bone

The system does not include : vascular endothelial cells or reticular cells & fibroblasts of lymphoid organs which may exhibit some phagocytic activity

Multinucleate giant cells → may form by fusion of macrophages or nuclear reduplication

(14)

Monocyte function → little function in circulating blood

(2)

(3) they migrate into the tissues & differentiate into macrophages in response to

- presence of necrotic material (necrotaxis)
- invading microorganisms (chemotaxis)
- inflammation

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↓

- have great capacity for phagocytosis
- large content of hydrolytic enzymes

→ form an integral part of immunological mechanisms e.g ⟨Antigen presentation⟩ (final destruction of antigen)

⇓

lymphocyte activation results in the production of factors which enhance macrophage phagocytic activity

<Hemopoiesis>
||
<Haematopoiesis>

Prenatally

1. Mesoblastic phase → in mesoderm of yolk sac
   (2nd - 6th) weeks of pregnancy
2. Hepatic phase → within liver & spleen also play a role
   ↓
   In the second trimester
3. myeloid phase → (in bone marrow)
   begins in the 3rd trimester

Post-natally

↓

occurs almost exclusively in RED bone marrow :-
sternum, Ribs, Pelvis
Proximal ends of femur
vertebrae

Bhorami

Pluripotent Hemopoietic Stem cells

It is believed that ALL BLOOD CELLS arise from a single type of stem cell in the bone marrow called a PLURIPOTENT STEM CELL

↓

it can produce ALL BLOOD CELL TYPES

proliferate & form TWO MAJOR CELL LINEAGES :

- → one for lymphoid cells (lymphocytes)
- → another for myeloid cells
  - → granulocytes
  - → monocytes
  - → erythrocytes
  - → megakaryocytes

} develop in bone marrow

Early in their development lymphoid cells migrate from the bone marrow to thymus or to the lymph nodes, spleen and other lymphoid structures where they proliferate & differentiate.

Progenitor & Precursor cells

(16) A

The Pluripotent Stem cells → give rise to daughter cells with restricted potentials called :

Progenitor cells (CFUs) or colony-forming units → CFUs

(4) types

* Since they give rise to colonies of ONLY ONE CELL TYPE when cultured or injected into spleen

A) Erythroid lineage of CFU-erythrocytes (CFU-E)

B) Thrombocytic >> >> CFU-megakaryocyte (CFU-Meg)

C) Granulocyte-monocyte lineage of CFU-granulocyte-monocytes (CFU-GM)

D) lymphoid lineage of CFU-lymphocytes of all types (CFU-L)

ALL (4) progenitor/CFUs produce → PRECURSOR cells or Blasts

"Bustani"

morphologic characteristics begins to differentiate suggesting the mature cell types they will become

STEM CELLS, PROGENITOR CELLS

cannot be morphologically distinguished & resemble LARGE LYMPHOCYTE

* Rate of cell division < slow in stem cells / Rapid in < progenitor, precursor > cells

* progenitor cells → can divide & produce both < Progenitor cells, Precursor >>

* Precursor cells → produce only mature blood cells

(16) B

Table 13–1. Changes in properties of hematopoietic cells during differentiation.

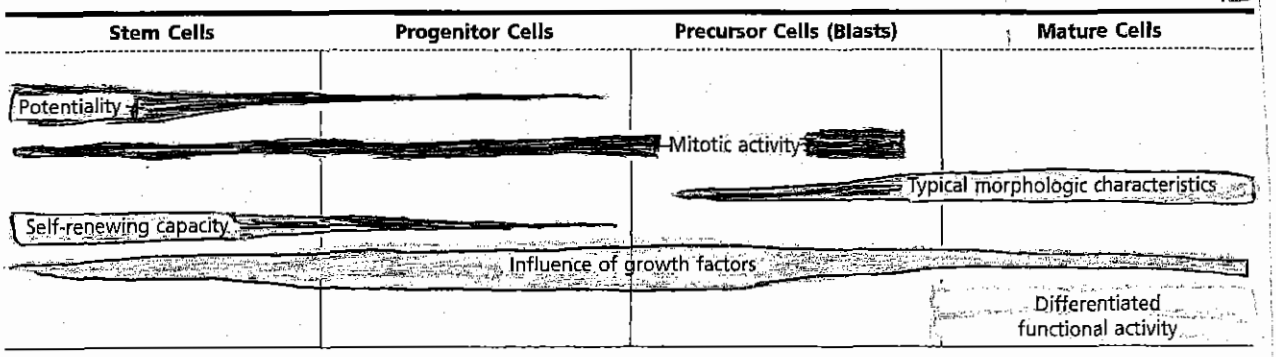

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Table 13–2. Main characteristics of five hemopoietic growth factors (colony-stimulating factors, CSF).

Name	Human Gene Location and Producing Cells	Main Biologic Activity
Granulocyte (G-CSF)	Chromosome 17 macrophages Endothelium fibroblasts	Stimulates formation (*in vitro* and *in vivo*) of granulocytes. Enhances metabolism of granulocytes. Stimulates malignant (leukemic) cells.
Granulocyte + macrophage (GM-CSF)	Chromosome 5 T lymphocytes Endothelium fibroblasts	Stimulates *in vitro* and *in vivo* production of granulocytes and macrophages.
Macrophage (M-CSF)	Chromosome 5 macrophages Endothelium fibroblasts	Stimulates formation of macrophages *in vitro*. Increases antitumor activity of macrophages.
Interleukin 3 (IL-3)	Chromosome 5 T lymphocytes	Stimulates *in vivo* and *in vitro* production of all myeloid cells.
Erythropoietin (EPO)	Chromosome 7 renal interstitial cells (outer cortex)	Stimulates red blood cell formation *in vivo* and *in vitro*.

Haemopoietic growth factors (colony stimulating factors) are proteins with complex overlapping functions → they

Stimulate
- proliferation of immature cells (mostly progenitor & precursor cells)
- support differentiation of maturing cells
- enhancing functions of mature cells

} may occur in the same growth factor or in different factors

Act as:
→ endocrine
→ paracrine
{from stromal cells of bone marrow}

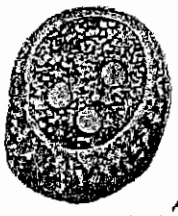
Potential therapeutic uses of growth factors

* After chemotherapy or irradiation → low blood count
* increasing the efficiency of marrow transplants by stimulating cell proliferation

→ Erythropoietin → activates cells of the erythrocytic series
→ thrombopoietin → stimulates platelets production
→ Steel factor (stem cell factor) → produced by stromal cells of bone marrow & acts on < multipotential / pluripotential cells

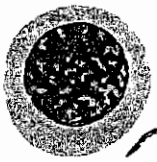
Erythropoiesis (Red cell formation)

- from stem cell to erythrocyte takes about 1 week
- rate is controlled by the hormone erythropoietin (17) secreted by the Kidney & by the availability of iron folic acid & vit. B_{12} & protein precursors



Proerythroblast

- the first recognizable erythrocyte precursor
- numerous organelles but No hemoglobin
- 15-20 μm diameter, basophilic cytoplasm finely granular chromatin, prominent nucleoli
- Further stages of differentiation are characterized by: (1) decreasing cell size (2) progressive loss of organelles !! the presence of numerous ribosomes at early stages account for the marked cytoplasmic basophilia (blue staining) → steadily decreases (3) progressive increase in hemoglobin content → account for increasing eosinophilia (Pink staining)



Basophilic erythroblast (Early normoblast)

- 12-16 μm diameter, deeply basophilic cytoplasm
- coarse chromatin, No nucleoli
- hemoglobin synthesis begins & is complete by the end of reticulocyte stage
- cell division ceases
- marks the beginning of progressive loss of cytoplasmic organelles

Polychromatophilic erythroblast (intermediate normoblast)

- 10-12 μm diameter
- cytoplasm varies from "blue-grey to slate-grey"
- Nucleus small with denser more compact chromatin structure

Orthochromatophilic erythroblast (late normoblast)

- Acidophilic erythroblast !! the cells have acquired almost their contents of hemoglobin & the cytoplasm become eosinophilic (Pink)
- small pyknotic nucleus
- nucleus extruded during this stage ??
- → Karyolysis (intracellular dissolution of nucleus)
- → Karyorrhexis (extrusion of nucleus) → phagocytosed by perisinusoidal macrophages

Reticulocyte

- slightly larger than mature erythrocyte
- acidophilic cytoplasm contains remnants of ribonucleoprotein stainable (brilliant cresyl blue) as a network
- may appear in circulating blood (Not more than 1%)
- → their number may increase after hemorrhage and in certain anemias

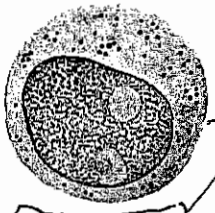
Erythrocyte

Granulopoiesis (granulocyte formation)

(18) A

Myeloblast

- most immature recognizable cell in the myeloid series
- light blue cytoplasm with NO granules
- nucleus
  - fine chromatin network
  - 2-3 pale nucleoli are evident

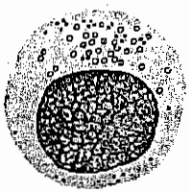


Promyelocyte

- basophilic (blue) cytoplasm with small dark azurophilic granules
- nucleus → Reddish blue
  - → coarse chromatin
  - nucleolus → present

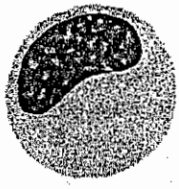
Early neutrophilic myelocyte

* Early basophilic myelocyte



Early eosinophilic myelocyte

- specific (secondary) granules gradually increase in quantity & eventually occupy most of the cytoplasm, with further condensation of nucleus



Late neutrophilic myelocyte

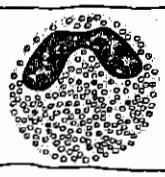
Late eosinophilic myelocyte

* Late basophilic myelocyte

Neutrophilic metamyelocyte

- cytoplasm → paler in colour
- nucleus → Kidney-shaped
  - → eccentric
  - dense, dark chromatin
  - absent nucleoli

Band cell (stab)



Eosinophilic metamyelocyte

- cytoplasm → more blue than a mature neutrophil, both azurophilic & specific granules are present
- nucleus → horseshoe-shaped dark blue with a very coarse granule
  - absent nucleoli

Mature neutrophil

Mature eosinophil

Mature basophil

→ Enters the blood stream :-

1. some appears to CIRCULATE
2. Others become adherent to endothelial cells of small vessels (Marginated) pool

* Entering the circulating pool in response to exercise & stress

* Bone marrow → contains a huge pool of stored neutrophils which can be rapidly mobilized should the need arise ⟹ corticosteroids increase the rate of release from the bone marrow

Recall: Azurophilic granules < Primary granules !! / large lysosomes

The appearance of large numbers of immature neutrophils (band cells) in the blood is called a **shift to the left** and is clinically significant, usually indicating bacterial infection.

Red bone marrow

- **Stroma** → meshwork of reticular cells & reticular fibres containing haemopoietic cells & macrophages
  - contains type I & III collagen
- **Sinusoidal capillaries** (fenestrated)
  - continuous endothelium & discontinuous basement membrane
  - Some regions of the endothelium are thin (little more than the inner & outer layers of plasma membrane) → Sites for migration of mature cells from Stroma → sinusoid → blood
- **Haemopoietic cords**
- **Releasing factors** !!
  - * C_3 components of complement
  - * hormones < glucocorticoids, androgens
  - * bacterial toxins !!

Recall → There are two types of **bone marrow** < Red, Yellow

- Red bone marrow → abundance of blood & hemopoietic cells
- Yellow bone marrow → filled with adipocytes
- In the newborn → ALL BONE MARROW IS RED & active in blood cell production

⇩

As the child grows most of the marrow changes gradually to the yellow variety

- under certain conditions < severe bleeding, hypoxia } yellow marrow reverts to Red
- Reticular cells of bone marrow → also called adventitial cells → produce steel factor (stem cell factor) that promote hemopoiesis through a paracrine effect

Ofusrami

Figure 7-1
Section of red marrow (low power).

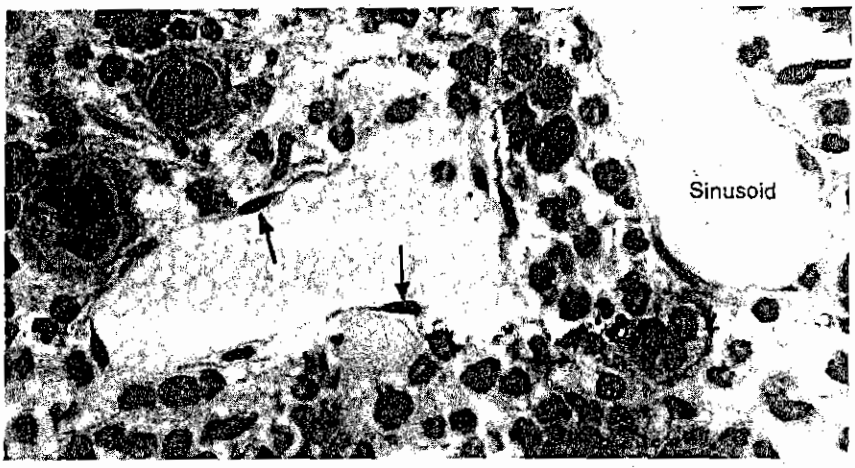
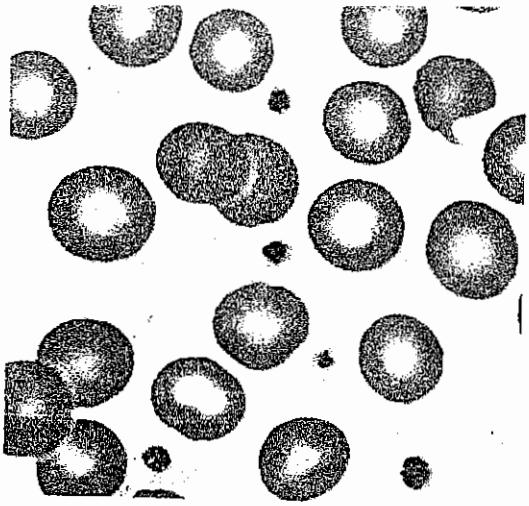


Figure 7-2
Sinusoids in red marrow (mouse), seen after experimental depletion of hematopoietic cells. Arrows indicate nuclei of fenestrated endothelial cells that line the sinusoids.

Functions of the Red bone marrow:

1. Production of blood cells
2. destruction of red blood cells (along with spleen & liver → removal of aged & defective erythrocytes from the circulation.
3. plays a central role in the immune system, being (a) the site of B lymphocyte differentiation (the mammalian equivalent of the bursa of Fabricius of birds) (b) containing large numbers of antibodies-secreting plasma cells



(a)

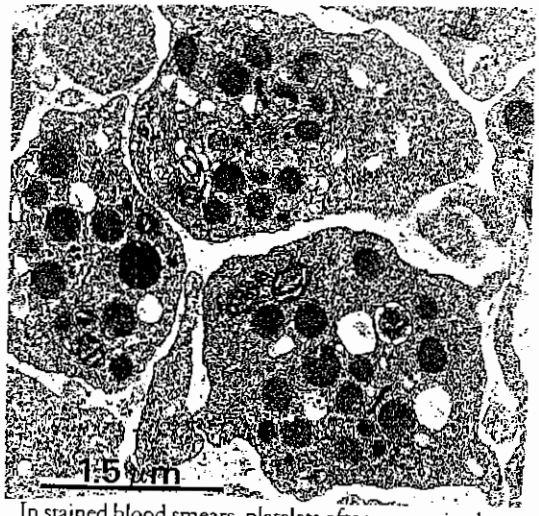
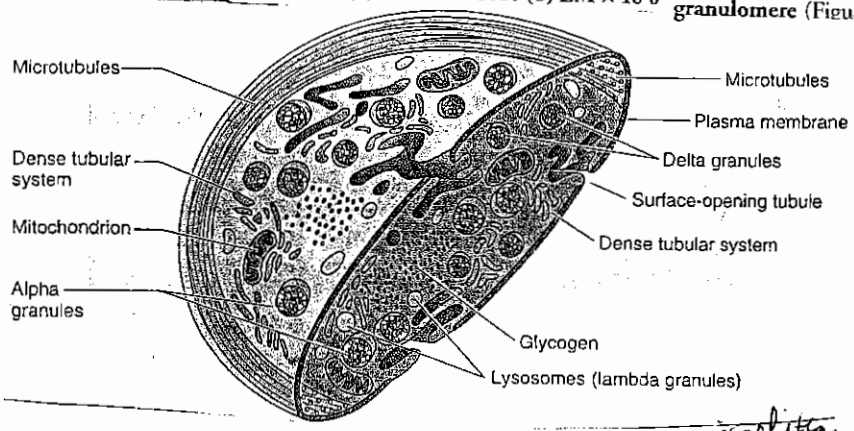


Fig. 3.10 Platelets (a) Giemsa × 1600 (b) EM × 18 0

In stained blood smears, platelets often appear in clumps. Each platelet has a lightly stained peripheral zone, the **hyalomere,** and a central zone containing darker-staining granules, called the **granulomere** (Figure 12–13).



Platelets (thrombocytes)

Small, non-nucleated cells formed in the bone marrow by fragmentation of the cytoplasm of mature megakaryocyte which in turn arise by differentiation of megakaryoblast

- Number : 150.000 – 400.000 / microlitre
- shape : biconvex discs 1.5–3.5 μm
- In blood film (smear) : Not clearly seen / often partially clumped together
- cytoplasm — Purple granular (numerous organelles)
  - hyalomere / granulomere — most conspicuous organelles → electron dense granules
- granules
  - alpha (largest) — contains : fibrinogen, factors V, VIII; platelet factor 4 → regulate vascular permeability
  - Dense (delta) — contain serotonin (absorped from plasma), ADP, ATP
  - lysosomes (lambda) → lysosomal enzymes → aid clot resorption
- cytoskeleton — beneath cell membrane : marginal band of microtubules & microfilaments
  - maintain ovoid shape
  - contractile protein — actin, myosin
- Dense tubular system (DTS) → deep to microtubules
  - function ?? synthesis of Prostaglandin
- Functions : haemostasis
  1) form plugs to occlude sites of vascular damage by adhering to collagen tissue at margin of wound → later replaced by fibrin
  2) Promote clot formation by providing surface for coagulation of protein complexes → thrombin formation

<Haemostasis>

↓

is the CONTROL & ARREST of BLEEDING

↓

depends on 3 interrelated factors

1- Reaction of blood vessel wall
2- Reaction of platelets
3- activation of blood coagulation mechanism

} A defect in any one may result in bleeding

Reaction of blood vessels

Injury to a blood vessel causes immediate local vasoconstriction accompanied by Reflex vasoconstriction of surrounding vessels ⇒ Reduction of blood flow to the injured area

→ lessens blood loss

→ assists the onset of platelet & coagulation activity

⇒ Bleeding depends:
- degree of injury
- size of vessels
- pressure of blood within vessels

⇒ when large vessels are injured → LOCAL COMPRESSION may be required to stop bleeding

Reaction of platelets

platelets form a PLUG at the site of damage to the vessel → ? during injury collagen fibres in the subendothelial connective tissue are exposed and PLATELETS ADHERE TO THEM → This adhesion depends on the Von willebrand Part of coagulation factor VIII in the plasma linking with a receptor glycoprotein on the membrane of platelets

As a result of adhesion → platelets release :

- Serotonin → constricts the blood vessels, maintaing & reinforcing the initial vasoconstriction & thus reducing blood flow
- adenosine diphosphate (ADP) → causes other platelets to aggregate on the already adhered platelets forming a plug which slows or stops bleeding

fibrin formation which is the end result of the blood coagulation process increases the stability of the plug

① The platelet membrane → contains phospholipids which on activation by collagen produce the prostaglandin derivative THROMBOXANE A_2

This substance is a potent aggregator of platelets and is also a powerful vasoconstrictor

② Vascular endothelial cells → synthesize another prostaglandin PROSTACYCLIN (PGI_2)

It is the most potent inhibitor of platelets aggregation yet discovered & is also a vasodilator

The opposing effects of these two prostaglandins BALANCE EACH OTHER so that in INTACT vessels there is normally NO PLATELETS AGGREGATION

Aspirin inhibits the synthesis of thromboxan A_2 by platelets & has an anticlotting effect

Blood coagulation

(23) Dr Bustami

- Soluble fibrinogen $\xrightarrow{\text{thrombin}^*}$ insoluble **fibrin mesh**

* thrombin → a proteolytic enzyme which splits two pairs of polypeptides from fibrinogen so that the remaining molecules can polymerize to form a fibrin mesh around the platelet plug

↓

Blood cells become entangled in this mesh

↓

Factor XIII activated by thrombin in the presence of Calcium ions → **Stabilizes the fibrin**

* thrombin — is formed from an inactive precursor (Prothrombin) as a result of a linked series of enzymatic reactions involving other coagulation factors present in < blood / tissues

⇓

There are **2 pathways** leading to the conversion of Prothrombin to thrombin

↓

The (intrinsic) pathway involves only factors circulating in blood

↓

An abnormal surface e.g damaged endothelium

↓

activates factor XII

↓

activates factor XI

↓

activates factor IX

↓

In the presence of Calcium ions, platelets phospholipid & factor VIII → activates factor X

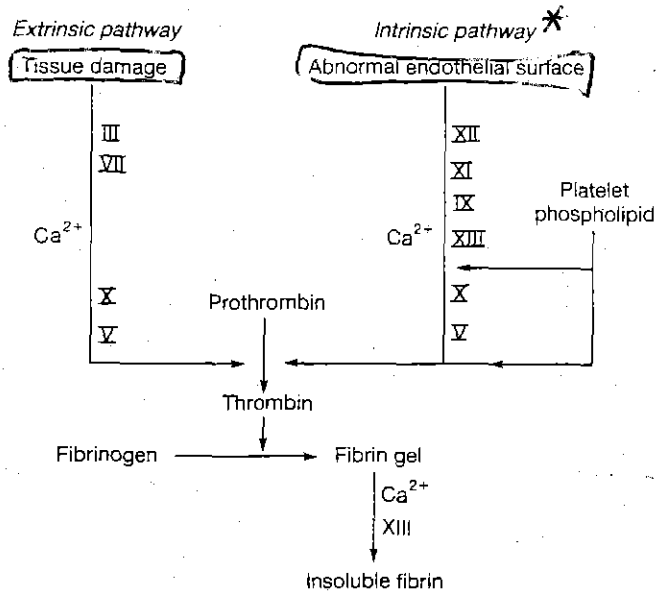
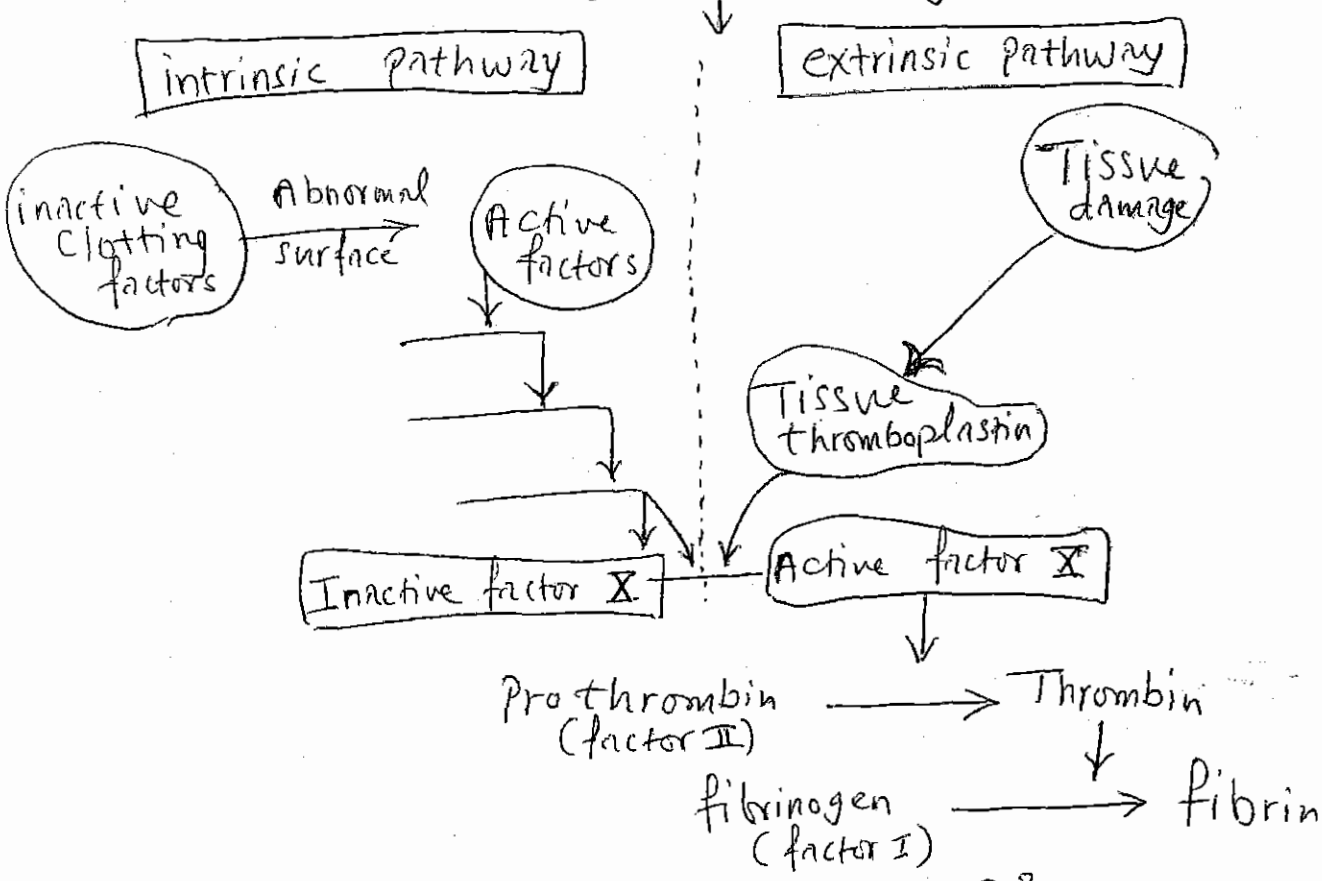


Fig. 10.7. Intrinsic and extrinsic pathways in blood coagulation

The extrinsic pathway is initiated when plasma is mixed with specific products of tissue damage collectively known as tissue thromboplastin → This BY-PASSES some of the initial steps in the intrinsic pathway

The two pathways CONVERGE, however at the ACTIVATION OF FACTOR X → the step prior to thrombin formation

Several steps in both pathways require Ca^{2+} which explains why Ca^{2+} chelating agents such as citrate are effective anticoagulants

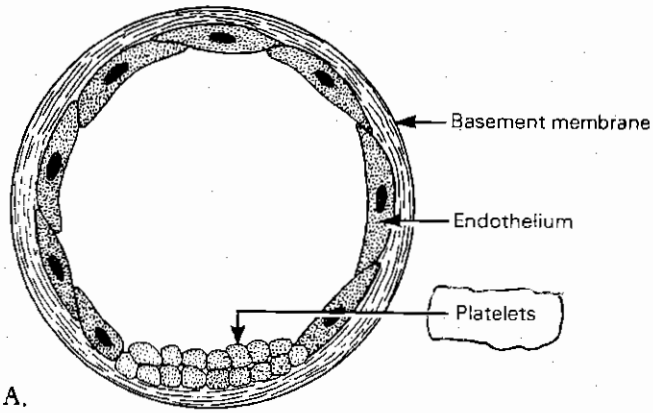


Recall: - Both pathways require Ca^{2+}
- the intrinsic pathway is accelerated by platelet factor 3 (PF3) from platelets

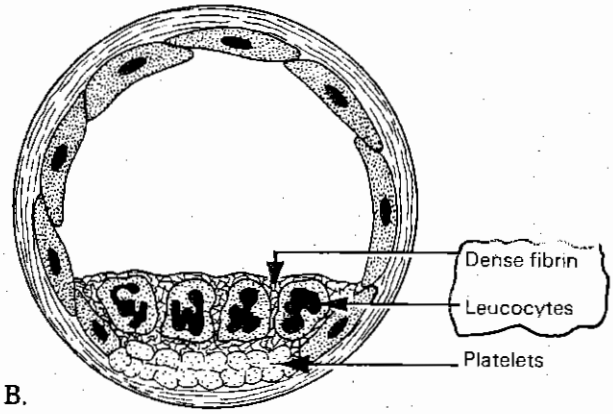
The (4) stages of thrombus formation :

(25)

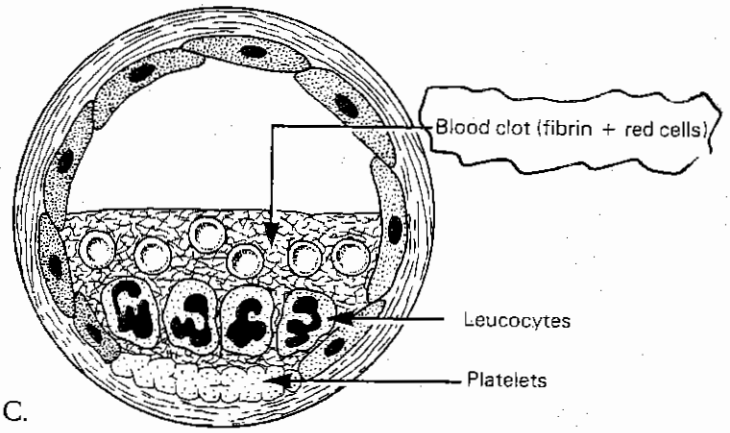
Obustani



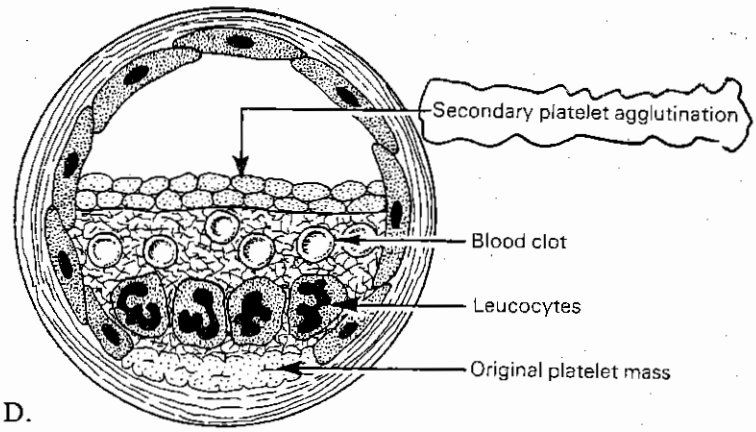
A.



B.



C.



D.

A. platelets stick to collagen or basement membrane

↓

B. Dense layer of fibrin & leucocytes adhere to the surface of platelets

↓

C. Blood Clot (fibrin, RBCs) form on the surface of leucocyte/platelet layer

↓

D. Fresh platelets agglutinate on surface of the blood clot

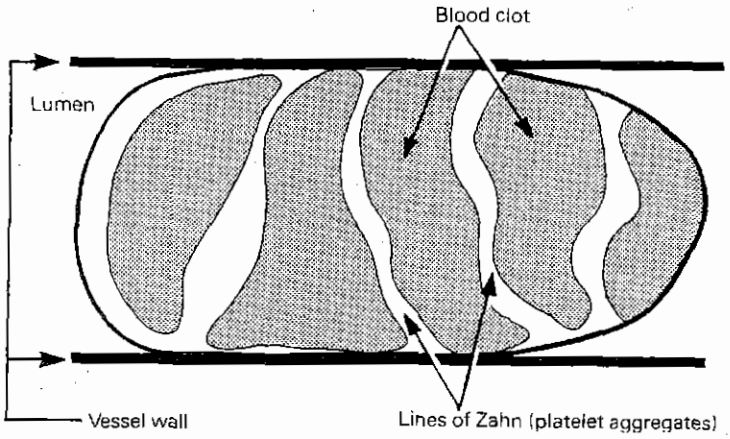
Remember :

① the composition of thrombi is similar in all types however venous thrombi have a higher proportion of blood clot (+++) (dark Red) to platelets (+)

② Arterial thrombi are best thought of simply as a complication of atheroma

③ venous thrombi occur usually in normal veins in patients immobilised in bed Recovering from operation or from childbirth or suffering from heart failure (DVT) !!!

Obstruction



20.2 Naked eye appearance of an occluding thrombus.

A thrombus is a solid mass formed from blood components in the living circulation. It is normally dry and firm and adherent to the wall of the vessel in which it has developed.

⇓

Remember that haemostasis (arrest of haemorrhage) consists essentially of 2 processes → coagulation being one, the other being composed of at least some of the stages of thrombosis

⇓

Thrombosis → begins with the ADHESION of platelets to the vessel wall and their clumping together to form a plug ⟹ This plug helps to arrest bleeding and may thereby save our lives but it is also the nucleus of thrombus formation, which may be fatal

⇓

Thrombus → striated appearance (lines of Zahn)
- striations → pale → platelet masses
- between striations → Red → blood clot ← fibrin, RBCs, WBCs

Thrombus
- occluding → in veins and arteries
- mural → in chambers of the heart & aorta
- vegetations → on heart valve following endocarditis

Fibrinolytic System

? محاضرة

(27)

- Fibrin is eventually broken down by a proteolytic enzyme → plasmin

- found in inactive precursor Plasminogen in blood ↕ tissue fluid

- Converted to plasmin by activators present in endothelium & tissues as well as by factor XII

There is almost certainly a slow release of plasminogen activator from capillaries / venules

Keeping these small vessels free from fibrin deposits

Plasmin like thrombin is a potent proteolytic enzyme → Its action is limited by

ANTIPLASMIN

circulating in the blood

— Anticoagulants —

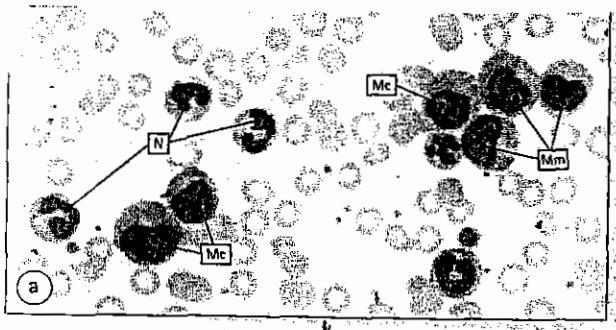
- citrate
- oxalate
- EDTA

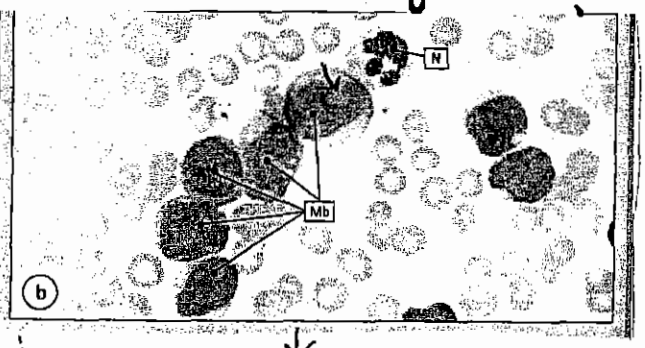
} Remove Ca^{++} from the blood

- Heparin
  - very potent anticoagulant
  - has direct antithrombotic action
  - prevents the conversion of prothrombin to thrombin
  - action lasts 6–8 hours

Warfarin & Phenindione
- suppress the synthesis of vit. K dependent factors (II, VII, IX & X)
- widely used in clinical practice
- action lasts for 48 hours.

→ Blood film ← Obusrami





Blood film from a Patient with Chronic granulocytic leukemia → increased number of MATURE WBCs mainly neutrophils (N) as well as PRECURSORS mainly

- myelocyte (Mc)
- metamyelocyte (Mm)

} escaped from bone marrow into blood

Blood film from a patient with an Acute granulocytic leukemia

↓

the malignant cells are immature granulocyte precursors

↓

mainly myeloblast (Mb)

↓

very few mature neutrophil (N) are being formed (arrow → nucleolus)

WHITE BLOOD CELL ABNORMALITIES

Increased numbers of white cells appear in the peripheral blood in a variety of disorders and provide a useful clue to underlying disease. For example, while in both cases the white cells are qualitatively normal, there is:—

- a considerable and sustained increase of circulating neutrophils in bacterial infections;
- an increase of circulating eosinophils in parasitic infestations and some allergies.

leukemia → malignant proliferation of white cell precursors in the bone marrow

↓

vast number of white cells & their precursors → many of which spell over into the blood

classification → According to the cell line involved (i.e granulocytic, monocytic, lymphocytic) ⊕ degree of malignancy

In chronic leukemias

↓

cells are partly or completely differentiated

e.g myelocytes, metamyelocytes band cells & neutrophils → disease → slowly progressive

In acute leukemia

↓

the proliferating cells are undifferentiated precursor cells

e.g myeloblasts in acute granulocytic leukemia & lymphoblasts in acute lymphatic leukemia.

→ Rapidly progressive